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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2  Apr 08   "Ask CAS" for self-help around the clock
 NEWS  3  Apr 09   BEILSTEIN: Reload and Implementation of a New Subject Area
 NEWS  4  Apr 09   ZDB will be removed from STN
 NEWS  5  Apr 19   US Patent Applications available in IFICDB, IFIPAT, and
IFIUDB
 NEWS  6  Apr 22   Records from IP.com available in CAPLUS, HCAPLUS, and
ZCAPLUS
 NEWS  7  Apr 22   BIOSIS Gene Names now available in TOXCENTER
 NEWS  8  Apr 22   Federal Research in Progress (FEDRIP) now available
 NEWS  9  Jun 03   New e-mail delivery for search results now available
 NEWS 10  Jun 10   MEDLINE Reload
 NEWS 11  Jun 10   PCTFULL has been reloaded
 NEWS 12  Jul 02   FOREGE no longer contains STANDARDS file segment
 NEWS 13  Jul 22   USAN to be reloaded July 28, 2002;
                   saved answer sets no longer valid
 NEWS 14  Jul 29   Enhanced polymer searching in REGISTRY
 NEWS 15  Jul 30   NETFIRST to be removed from STN
 NEWS 16  Aug 08   CANCERLIT reload
 NEWS 17  Aug 08   PHARMAMarketLetter(PHARMAML) - new on STN
 NEWS 18  Aug 08   NTIS has been reloaded and enhanced
 NEWS 19  Aug 19   Aquatic Toxicity Information Retrieval (AQUIRE)
                   now available on STN
 NEWS 20  Aug 19   IFIPAT, IFICDB, and IFIUDB have been reloaded
 NEWS 21  Aug 19   The MEDLINE file segment of TOXCENTER has been reloaded
 NEWS 22  Aug 26   Sequence searching in REGISTRY enhanced
 NEWS 23  Sep 03   JAPIO has been reloaded and enhanced
 NEWS 24  Sep 16   Experimental properties added to the REGISTRY file
 NEWS 25  Sep 16   CA Section Thesaurus available in CAPLUS and CA
 NEWS 26  Oct 01   CASREACT Enriched with Reactions from 1907 to 1985
 NEWS 27  Oct 21   EVENTLINE has been reloaded
 NEWS 28  Oct 24   BEILSTEIN adds new search fields
 NEWS 29  Oct 24   Nutraceuticals International (NUTRACEUT) now available on
STN
 NEWS 30  Oct 25   MEDLINE SDI run of October 8, 2002
 NEWS 31  Nov 18   DKILIT has been renamed APOLLIT
 NEWS 32  Nov 25   More calculated properties added to REGISTRY
 NEWS 33  Dec 02   TIBKAT will be removed from STN
 NEWS 34  Dec 04   CSA files on STN
 NEWS 35  Dec 17   PCTFULL now covers WP/PCT Applications from 1978 to date
 NEWS 36  Dec 17   TOXCENTER enhanced with additional content
 NEWS 37  Dec 17   Adis Clinical Trials Insight now available on STN
 NEWS 38  Dec 30   ISMEC no longer available
 NEWS 39  Jan 21   NUTRACEUT offering one free connect hour in February 2003
 NEWS 40  Jan 21   PHARMAML offering one free connect hour in February 2003
 NEWS 41  Jan 29   Simultaneous left and right truncation added to COMPENDEX,
                   ENERGY, INSPEC
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NEWS 42 Feb 13 CANCERLIT is no longer being updated
NEWS 43 Feb 24 METADEX enhancements
NEWS 44 Feb 24 PCTGEN now available on STN
NEWS 45 Feb 24 TEMA now available on STN
NEWS 46 Feb 26 NTIS now allows simultaneous left and right truncation
NEWS 47 Feb 26 PCTFULL now contains images
NEWS 48 Mar 04 SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 49 Mar 19 APOLLIT offering free connect time in April 2003
NEWS 50 Mar 20 EVENTLINE will be removed from STN
NEWS 51 Mar 24 PATDPAFULL now available on STN
NEWS 52 Mar 24 Additional information for trade-named substances without structures available in REGISTRY
NEWS 53 Mar 24 Indexing from 1957 to 1966 added to records in CA/CAPLUS

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=> s PCr (2a) hybridization (s) primers
L1 1385 PCR (2A) HYBRIDIZATION (S) PRIMERS

=> s l1 and multiplex
L2 45 L1 AND MULTIPLEX

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 26 DUP REM L2 (19 DUPLICATES REMOVED)

=> d l3 ti so au

L3 ANSWER 1 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI **Multiplex** PCR-microarray hybridization method for simultaneous detection of nucleic acid molecules
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
IN Schmidt, Wolfgang; Mundlein, Axel; Huber, Martin; Kroath, Hans

=> d l3 2-26 ti so au

L3 ANSWER 2 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Detection of EHEC by hybridization or PCR analysis of Slt and eae and/or hlyA loci
SO PCT Int. Appl., 86 pp.
CODEN: PIXXD2
IN Grabowski, Reiner; Groenewald, Cordt; Schneider, Astrid; Pardigol, Andreas; Berghof, Kornelia

L3 ANSWER 3 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Estimation of genetic divergence among elite cotton cultivars-genotypes by
DNA fingerprinting technology
SO Crop Science (2002), 42(6), 2137-2144
CODEN: CRPSAY; ISSN: 0011-183X
AU Rahman, M.; Hussain, D.; Zafar, Y.

L3 ANSWER 4 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Bordetella pertussis PCR: simultaneous targeting of signature sequences
SO Diagnostic Microbiology and Infectious Disease (2002), 43(4), 269-275
CODEN: DMIDDZ; ISSN: 0732-8893
AU Qin, Xuan; Turgeon, David K.; Ingersoll, Brian P.; Monsaas, Peter W.; Lemoine, Christina J.; Tsosie, Treva; Stapp, Lynn O.; Abe, Patrick M.

L3 ANSWER 5 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI A novel **multiplex** RT-PCR probe capture hybridization (RT-PCR-ELISA) for simultaneous detection of six viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid, and Pospiviroid
SO Journal of Virological Methods (2002), 105(1), 115-121
CODEN: JVMEDH; ISSN: 0166-0934
AU Shamloul, A. M.; Faggioli, F.; Keith, J. M.; Hadidi, A.

L3 ANSWER 6 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Multiple-site reaction device and method for sequence-specific nucleic acid targeting
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
IN Sharat, Singh; Cao, Liching; Hooper, Herbert H.; Albagli, David; Anderson,
Rolfe; Zeng, Shulin

L3 ANSWER 7 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
TI Solid-phase PCR with hybridization and time-resolved fluorometry for detection of HLA-B27.
SO Clinical Chemistry, (March, 2001) Vol. 47, No. 3, pp. 498-504. print.
ISSN: 0009-9147.
AU Sjoroos, Minna (1); Ilonen, Jorma; Lovgren, Timo

L3 ANSWER 8 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Rapid detection of enterohemorrhagic Escherichia coli by real-time PCR with fluorescent hybridization probes
SO Journal of Clinical Microbiology (2001), 39(1), 370-374
CODEN: JCMIDW; ISSN: 0095-1137
AU Bellin, Tobias; Pulz, Matthias; Matussek, Andreas; Hempen, Hans-Gunther; Gunzer, Florian

L3 ANSWER 9 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Direct detection of vancomycin-resistant enterococci from 553 fecal samples during an hospital outbreak using **multiplex** PCR and capture probe hybridization.
SO Abstracts of the General Meeting of the American Society for Microbiology,
(2001) Vol. 101, pp. 152.
http://www.asmusa.org/mtgsrc/generalmeeting.htm.
print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
AU Leclerc, B. (1); Huletsky, A. (1); Bernal, A. (1); Maynard, C. (1); Clairoux, N. (1); Picard, F. J. (1); Dion, M. (1); Gagnon, M. (1); Gagnon,
F. (1); Trottier, S.; Brochu, G.; Ouellette, M. (1); Roy, P. H. (1); Bergeron, M. G. (1)

L3 ANSWER 10 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Detecting and genotyping Escherichia coli O157:H7 using multiplexed PCR and nucleic acid microarrays
SO International Journal of Food Microbiology (2001), 67(1-2), 71-80
CODEN: IJFMDD; ISSN: 0168-1605
AU Call, D. R.; Brockman, F. J.; Chandler, D. P.

L3 ANSWER 11 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Deletion and duplication analysis in males affected with Duchenne or Becker muscular dystrophy
SO Methods in Molecular Medicine (2001), 43(Muscular Dystrophy), 53-84
CODEN: MMMEFN
AU Curtis, Ann; Haggerty, Daisy

L3 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI **Multiplex** PCR-enzyme hybridization assay using unequal primer concentrations to detect human parainfluenza virus 1, 2, 3 and respiratory

syncytial virus A, B and influenza virus A, B
SO U.S., 26 pp., Cont.-in-part of U. S. 5,744,299.
CODEN: USXXAM
IN Henrickson, Kelly J.; Fan, Jiang

L3 ANSWER 13 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Real-time **multiplex** PCR for the quantitation of t(14;18) associated with follicular lymphoma.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 222b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
AU Choppa, Paul (1); Gomez, Jamie (1); Vall, Horacio (1); Owens, Marilyn (1);
Lopategui, Jean (1)

L3 ANSWER 14 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI PCR detection of Fusarium oxysporum f.sp. gladioli race 1, causal agent of
Gladiolus yellows disease, from infected corms.
SO Plant Pathology (Oxford)., (Jan., 2000) Vol. 49, No. 1, pp. 89-100.
ISSN: 0032-0862.
AU de Haan, L. A. M. (1); Numansen, A.; Roebroeck, E. J. A.; van Doorn, J.

L3 ANSWER 15 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
TI A **multiplex** reverse transcription polymerase chain reaction method for the detection of foodborne viruses.
SO Journal of Food Protection, (Oct., 1999) Vol. 62, No. 10, pp. 1210-1214.
ISSN: 0362-028X.
AU Rosenfield, Soraya I.; Jaykus, Lee-Ann (1)

L3 ANSWER 16 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
TI Population genetic data on loci LDLR, GYPA, HBGG, D7S8 and GC in the Bangkok population compared with rural Thais from Trat province.
SO Journal of the Medical Association of Thailand, (Aug., 1999) Vol. 82, No. 8, pp. 784-791.
ISSN: 0125-2208.
AU Sueblinvong, Tada (1); Anomasiri, Wilai (1); Sueblinvong, Tanasak; Sirisup, Nantana; Kongsrisook, Unchalee (1)

L3 ANSWER 17 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
TI Detection of clinical vancomycin-resistant enterococci in Denmark by **multiplex** PCR and sandwich hybridization.
SO APMIS, (April, 1999) Vol. 107, No. 4, pp. 404-412.
ISSN: 0903-4641.
AU Poulsen, Rikke Lykke (1); Pallesen, Lars V.; Frimodt-Moller, Niels; Espersen, Frank

L3 ANSWER 18 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
TI Simultaneous detection of erythromycin-resistant methylase genes ermA and ermC from Staphylococcus spp. by **multiplex**-PCR.

SO Molecular and Cellular Probes, (Oct., 1999) Vol. 13, No. 5, pp. 381-387. ISSN: 0890-8508.
AU Khan, S. A.; Nawaz, M. S. (1); Khan, A. A.; Cerniglia, C. E.

L3 ANSWER 19 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Detection of Treponema pallidum, Haemophilus ducreyi, and herpes simplex virus by **multiplex** PCR
SO Methods in Molecular Medicine (1999), 20(Sexually Transmitted Diseases), 67-79
CODEN: MMMEFN
AU Orle, Karina A.; Weiss, Judith B.

L3 ANSWER 20 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
TI Rearranged immunoglobulin light chain genes as minimal residual disease markers in intermediate- and high-grade malignant B cell non-Hodgkin's lymphoma.
SO Leukemia (Basingstoke), (Nov., 1998) Vol. 12, No. 11, pp. 1810-1816. ISSN: 0887-6924.
AU Hoogeveen-Westerveld, M.; Hupkes, P. E.; Doekharan, D.; Dorssers, L. C. J.; Van't Veer, M. B. (1); Van Belzen, N.

L3 ANSWER 21 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
TI Detection of microbial pathogens in shellfish with **multiplex** PCR.
SO Current Microbiology, (Aug., 1998) Vol. 37, No. 2, pp. 101-107. ISSN: 0343-8651.
AU Brasher, Cynthia W.; Depaola, Angelo; Jones, Daniel D.; Bej, Asim K. (1)

L3 ANSWER 22 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Use of polymerase chain reaction and serum antibodies for diagnosis of enterohemorrhagic Escherichia coli.
SO Journal of the Korean Society for Microbiology, (Feb., 1998) Vol. 33, No. 1, pp. 99-110. ISSN: 0253-3162.
AU Kim, Yung-Bu (1); Oh, Yang-Hyo

L3 ANSWER 23 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Monitoring hybridization during PCR with rapid thermal cycling and fluorophore-labeled probes
SO PCT Int. Appl., 216 pp.
CODEN: PIXXD2
IN Wittwer, Carl T.; Ririe, Kirk M.; Rasmussen, Randy P.

L3 ANSWER 24 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Genetic detection of human hemoglobin .alpha. gene deletions, .alpha.-thalassemia mutations, and their use as predictors of blood-related disorders such as hypertension
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
IN Bowie, Lemuel J.

L3 ANSWER 25 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
TI An optimized **multiplex** polymerase chain reaction (PCR) for detection of BCR-ABL fusion mRNAs in haematological disorders.

SO Leukemia (Basingstoke), (1994) Vol. 8, No. 1, pp. 186-189.
ISSN: 0887-6924.
AU Cross, Nicholas C. P. (1); Melo, Junia V.; Feng, Lin; Goldman, John M.

L3 ANSWER 26 OF 26 CAPLUS COPYRIGHT 2003 ACS
TI Detection of human papillomavirus types 6, 11, 16 and 18 in mucosal and cutaneous lesions by the **multiplex** polymerase chain reaction
SO Journal of Virological Methods (1991), 35(2), 143-57
CODEN: JVMEDH; ISSN: 0166-0934
AU Soler, C.; Allibert, P.; Chardonnet, Y.; Cros, P.; Mandrand, B.; Thivolet, J.

=> d l3 1, 10, 12, abs

L3 ANSWER 1 OF 26 CAPLUS COPYRIGHT 2003 ACS
AB A method is disclosed for the simultaneous detection of at least two mutually different nucleic acid mols. in a sample. In a first step a **multiplex** PCR and in a second step a hybridization reaction are carried out on immobilized probes in a microarray. The hybridized PCR products are then detected and optionally quantified, whereby the probes applied for the hybridization reaction, which each hybridize specifically with nucleic acids which are different from each other, have m.ps. which differ from each other by at most 2 .degree.C, and preferably 1 .degree.C.
Thus, using Gene Runner software, probes with Tm of 65.+-.1.degree. were designed for PBP2, KanR, MecR, DhfrA, StrR, VanB, MlsR, AmpR, CmR, TetR, FosB, and AacA genes. Microarrays contg. these probes were then used to detect the microbial antibiotic resistance genes.

L3 ANSWER 10 OF 26 CAPLUS COPYRIGHT 2003 ACS
AB Rapid detection and characterization of food borne pathogens such as Escherichia coli O157:H7 is crucial for epidemiol. investigations and food safety surveillance. As an alternative to conventional technologies, we examd. the sensitivity and specificity of nucleic acid microarrays for detecting and genotyping E. coli O157:H7. The array was composed of oligonucleotide probes (25-30 mer) complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolysin A). Target DNA was amplified from whole cells or from purified DNA via single or multiplexed polymerase chain reaction (PCR), and PCR products were hybridized to the array without further modification or purifn. The array was 32-fold more sensitive than gel electrophoresis and capable of detecting amplification products from <1 cell equiv. of genomic DNA (1 fg). Immunomagnetic capture, PCR and a microarray were subsequently used to detect 55 CFU ml-1 (E. coli O157:H7) from chicken rinsate without the aid of pre-enrichment. Four isolates of E. coli O157:H7 and one isolate of O91:H2, for which genotypic data were available, were unambiguously genotyped with this array. Glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products; the system is amenable to automation.

L3 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2003 ACS
AB A PCR-based method for detecting the presence of multiple virus infections in a biol. sample, PCR-enzyme hybridization assay, or PCR-EHA is disclosed. In one embodiment, this method comprises the step of (1) isolating a nucleic acid either in the form of RNA or cDNA derived from

it, (2) exposing the nucleic acid to a primer pair derived from the sequences of human parainfluenza virus 1, 2 and 3, respiratory syncytial virus A and B and influenza virus, A and B to carry out a PCR amplification, and (3) examg. the amplification reaction product using the protein-linked oligonucleotide probes attached to a sold support. In another embodiment, the invention is an improved PCR method. where the 5' and 3' primers of unequal concns. are used to improve the detection.

=> d l3 12 ibib

L3 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:46953 CAPLUS
DOCUMENT NUMBER: 132:89219
TITLE: **Multiplex** PCR-enzyme hybridization assay using unequal primer concentrations to detect human parainfluenza virus 1, 2, 3 and respiratory syncytial virus A, B and influenza virus A, B
INVENTOR(S): Henrickson, Kelly J.; Fan, Jiang
PATENT ASSIGNEE(S): MCW Research Foundation, USA
SOURCE: U.S., 26 pp., Cont.-in-part of U. S. 5,744,299.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6015664	A	20000118	US 1996-691045	19960801
US 5744299	A	19980428	US 1995-552907	19951103
WO 9716570	A1	19970509	WO 1996-US17405	19961101
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML				
AU 9674838	A1	19970522	AU 1996-74838	19961101
AU 716222	B2	20000224		
EP 865503	A1	19980923	EP 1996-937087	19961101
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 2002081567	A1	20020627	US 2000-484704	20000118

PRIORITY APPLN. INFO.: US 1995-552907 A2 19951103
US 1996-691045 A 19960801
WO 1996-US17405 W 19961101

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1: Nucleic Acids Res 2002 Jan 15;30(2):E5

Related Articles, Links

A new class of homogeneous nucleic acid probes based on specific displacement hybridization.

Li Q, Luan G, Guo Q, Liang J.

The Key Laboratory of Cell Biology and Tumor Cell Engineering of the Ministry of Education, Xiamen 361005, Fujian, China. qing@phri.nyu.edu

We have developed a new class of probes for homogeneous nucleic acid detection based on the proposed displacement hybridization. Our probes consist of two complementary oligodeoxyribonucleotides of different length labeled with a fluorophore and a quencher in close proximity in the duplex. The probes on their own are quenched, but they become fluorescent upon displacement hybridization with the target. These probes display complete discrimination between a perfectly matched target and single nucleotide mismatch targets. A comparison of double-stranded probes with corresponding linear probes confirms that the presence of the complementary strand significantly enhances their specificity. Using four such probes labeled with different color fluorophores, each designed to recognize a different target, we have demonstrated that multiple targets can be distinguished in the same solution, even if they differ from one another by as little as a single nucleotide. Double-stranded probes were used in real-time nucleic acid amplifications as either probes or as primers. In addition to its extreme specificity and flexibility, the new class of probes is simple to design and synthesize, has low cost and high sensitivity and is accessible to a wide range of labels. This class of probes should find applications in a variety of areas wherever high specificity of nucleic acid hybridization is relevant.

PMID: 11788731 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1999 Sep 10;273(2):221-8

Color multiplexing hybridization probes using the apolipoprotein E locus as a model system for genotyping.

Bernard PS, Pritham GH, Wittwer CT.

Department of Pathology, University of Utah Medical School, 50 North Medical Drive, Salt Lake City, Utah 84132, USA.

Fluorescent hybridization probes were multiplexed for color genotyping of the apolipoprotein E locus using model oligonucleotide targets. Fluorescence resonance energy transfer was observed during adjacent hybridization of 3'-fluorescein-labeled "donor" probes paired with 5'-labeled "acceptor" probes with different emission spectra reporting at codons 112 and 158. The acceptor dyes emitted at either 640 nm (LightCycler Red 640) or 705 nm (LightCycler Red 705) and were monitored with a LightCycler, a thermal cycler with an integrated fluorimeter. The color of the acceptor dye identified each site and the characteristic melting temperatures of the fluorescein-labeled probes identified single base changes within each codon. Color compensation of temperature-dependent spectral overlap was applied to completely separate each channel. Competition between the probes and the complementary strand for the target sequence decreased resonance energy transfer, indicating an advantage of single-stranded target. Hybridization probes of the same length, but different GC content are T(m) shifted by the same amount during A:C mismatch duplex melting. Genotyping was optimal at both sites if melting curve analysis was preceded by a slow (1 degrees C/s) annealing phase. Although each site preferred different concentrations of Mg(2+) and target strand for optimal genotyping, conditions for multiplexing were found. This method, along with an appropriate amplification technique, should allow real-time multiplex genotyping from genomic DNA. Copyright 1999 Academic Press.

PMID: 10469493 [PubMed - indexed for MEDLINE]

1: J Clin Pathol 1998 Jan;51(1):38-43

DNA-EIA to detect high and low risk HPV genotypes in cervical lesions with E6/E7 primer mediated multiplex PCR.

Clavel C, Rihet S, Masure M, Chypre C, Boulanger JC, Quereux C, Birembaut P.

Unite de Biologie Cellulaire, Hopital de la Maison Blanche, CHU de Reims, France.

BACKGROUND: Oncogenicity of human papillomavirus (HPV) DNA in premalignant and malignant uterine cervical diseases is mainly induced by E6/E7 open reading frame (ORF). The presence of an oncogenic HPV DNA may be a diagnostic marker for the detection of cytologically negative smears. AIMS: To evaluate an original polymerase chain reaction enzyme immunoassay (PCR-EIA) for the detection and typing of oncogenic and non-oncogenic HPV types. METHODS: The test was an original multiplex labelled PCR-EIA for the detection and typing of oncogenic and non-oncogenic HPV using three consensus sequence primers within the oncogenic E6/E7 ORF. One primer was dinitrophenyl (DNP) labelled and the DNP labelled amplimers could be further hybridised with specific biotinylated oligoprobes mixed in only two cocktails: oncogenic (16, 18, 31, 33, 35, 52, and 58) and non-oncogenic (6 and 11) HPV types in only two wells; then biotinylated oligoprobes were deposited in streptavidin-coated microplates. The PCR-EIA was validated on HPV plasmids (types 6, 11, 16, 18, 31, 35, 52, and 58) and used to evaluate cervical scrapes from 181 patients (median age 32 years) at high risk for cervical cancer. RESULTS: HPV were detected in the cervical scrapes of 88 of 181 patients (48.6%); nine with non-oncogenic HPV (5.0%) and 79 with oncogenic HPV (43.6%) including 29 coinfections with oncogenic and non-oncogenic HPV. The number of oncogenic HPV infections increased with the presence of high grade lesions: 95.8% of the cervical scrapes from patients with high grade lesions contained oncogenic HPV compared with 32.1% of the specimens from patients without any lesions detectable by colposcopy and/or by cytological examination of the cervical smears. Moreover, 60% of cervical scrapes exhibiting low grade lesions contained oncogenic HPV. CONCLUSIONS: This test is simple, specific, sensitive, safe, fast, reproducible, and easy to use in routine practice. Thus, it is possible to detect simultaneously on a simple cervical scrape, two kinds of HPV--oncogenic and non-oncogenic--in just two microplate wells with non-isotopic oligoprobes.